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Intracellular Transport: Force Controls Motor Switching at Filament Junctions

How does cellular traffic switch direction on microtubules, or switch back and forth between microtubule- and actin-based tracks? A pair of recent studies mimics the layout of motors and tracks within the cell to provide intriguing answers to these questions.

Ronald S. Rock

"You are in a maze of twisty little passages, all alike."

Those of us who grew up in the early days of the personal computer will recognize that line from the text-based game *Adventure*, in which you explore a fantastic cave full of dwarves, dragons, and magical objects. The maze in that game resembled a twisted Roman street-plan, in that moving to the south was no guarantee that you would enter the next area from the north. The solution to this challenge was to drop objects to serve as markers, which would then allow you to map out the maze, fully explore it, and find the exit.

For the molecular motors that must navigate the cell, the challenge is far more difficult. They also face a maze of twisty little passages constructed from actin filaments and microtubules, but, unlike our virtual spelunker, they must navigate without a breadcrumb trail to guide them. The motility field commonly thinks of motors as the machines that organize the cell, setting a place for everything and everything in its place. However, we know remarkably little about how these motors decide when and where to turn within the cell [1]. That is now beginning to change, as a pair of studies published recently in *Current Biology* by Schroeder *et al.* [2] and Hendricks *et al.* [3] describes how cargoes may switch direction when transported by multiple types of motors.

Schroeder *et al.* [2] addressed the problem of how traffic is switched back and forth from microtubules to actin filaments, using small collections of cytoplasmic dynein and myosin V motors. Such handoffs between motor systems are common in biology. A classic example is found in the frog melanophore system. These remarkable cells can rapidly change color by collecting pigment granules near the cell center, or by dispersing these granules throughout the cell periphery. When the pigment granules return to the cell center, they must switch from myosin-V based transport along cortical actin filaments to dynein-based inward transport along microtubules [4–6]. How does a single granule decide whether to switch to the microtubule? The possibilities range from simple tug-of-war scenarios, where the strongest collection of motors will win [7], to complex mechanisms involving concerted inactivation of one motor and activation of the other [8,9].

To test whether mechanical signaling alone could lead to predictable track-switching behavior, Schroeder *et al.* [2] developed a clever *in vitro* system to mimic the traffic situation within cells [1]. These authors fixed actin filaments and microtubules to a coverslip and sought areas where the two filaments crossed. To mimic the cargo, they applied beads coated with 1–4 myosin V molecules and 1–4 dynein molecules at the junction. These beads were separately characterized in an optical trap to find the maximum force that they could develop along each track; somewhat surprisingly, the maximal forces are roughly additive, so that two motors on occasion will pull with twice the force of one [10]. Schroeder *et al.* [2] observed four types of bead behavior:

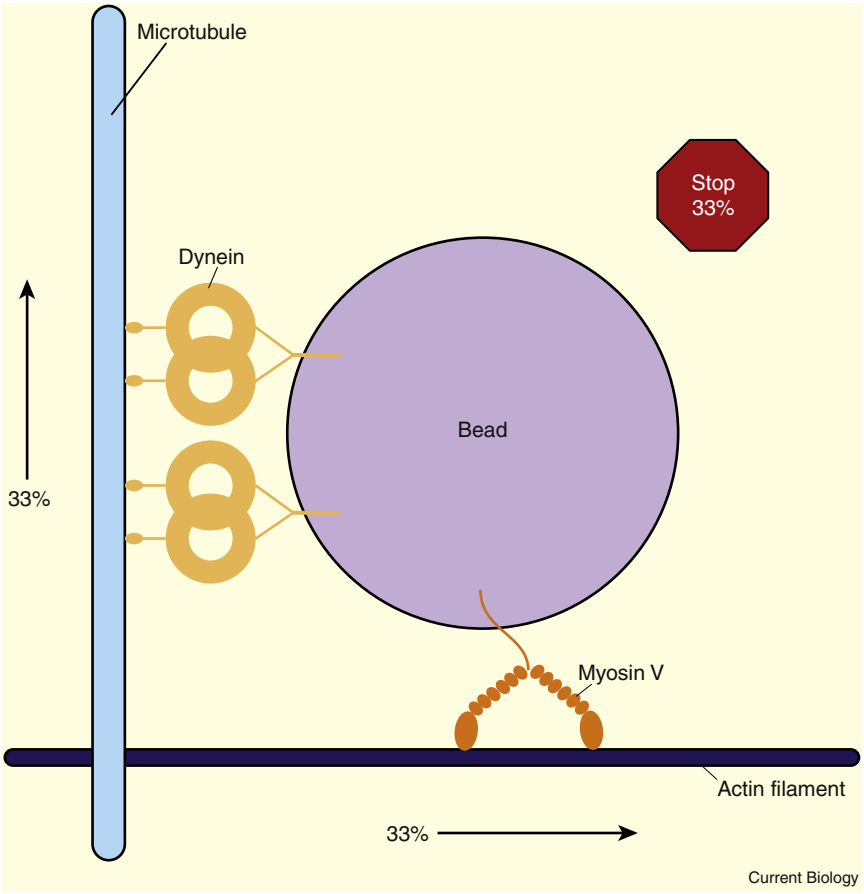

Figure 1. A tug-of-war determines the outcome when multiple motors engage cytoskeletal tracks.

A cartoon depicting the experimental scheme used by Schroeder *et al.* [2]. Actin filaments and microtubules are attached to a surface, using orthogonal flow to set up crossed, overlapping filaments. These filaments may be overlapped in either order, creating defined overpasses and underpasses. A bead coated with various amounts of dynein and myosin V is maneuvered with an optical trap on one of the two filaments near the intersection. As the bead moves through the intersection, it either continues on the same filament, switches to the other filament, pauses, or detaches (although detachment is rare). In the situation depicted in the cartoon, this bead contains two dynein motors and one myosin V motor. Since the dynein stall force is approximately half that of myosin V, and since the forces generated by each motor are approximately additive, the situation drawn here represents a stalemate, where the bead will stop at the intersection (observed ~33% of the time at the 2:1 molar ratio of dynein to myosin V). However, if some motors are detached from their filament when the bead reaches the intersection, the bead can either continue on the same filament (also observed 33% of the time) or switch to the other filament (33% of the time) in a manner determined by the force imbalance.

continuing along the initial track; switching to the crossed track; pausing at the intersection; and detaching when all motors release their tracks (Figure 1).

Remarkably, the winning motor–track combination could be largely predicted from the set of motors that would generate the greater maximal force. If myosin V pulls harder than the dynein on a given bead, the bead will continue along the actin filament (if it started along actin), or will switch to actin (if it started along the microtubule). In some cases, myosin V and dynein were equally matched, and the bead stalled at the intersection. Using this information, Schroeder *et al.* [2] then developed a simple binomial model that could predict the likelihood of continuing, switching, pausing, or detaching for each dynein:myosin V ratio. This model can account for cases in which beads that should produce more force from myosin V would nevertheless switch to the microtubule, given that some of the myosin V motors may be detached at the critical moment when the bead reaches the intersection.

The related paper by Hendricks *et al.* [3] examined the similar case of direction switching of vesicles transported along microtubules. They find a similar tug-of-war situation, but in this case driven by dynein and kinesin-2. Hendricks *et al.* [3] isolated neuronal vesicles from mice that expressed GFP-labeled dynactin. These isolated vesicles move along microtubules much like they do in the intact cells. However, isolation of the vesicles offered a considerable advantage over purely *in vivo* work, as Hendricks *et al.* [3] were able to determine the number of each type of motor on the vesicles. By measuring stepwise photobleaching, these authors were able to count the average number of dynactin molecules on the vesicles. Using quantitative Western blots and comparing protein abundance to the dynactin standard, they estimated the number of kinesin-1, kinesin-2, and dynein motors on the vesicles. All three motors were in the low single-digits, with about twice as many dynein motors as kinesin-2 motors. This is the perfect situation for bidirectional movement by stochastic binding and release of motors in a tug-of-war, and indeed this is what Hendricks *et al.* [3] observed both *in vitro* and *in vivo*. Furthermore, using the *in vitro* system, these authors were also able to control motor activity and ultimately vesicle direction with specific antibodies. For example, an antibody that blocks the activity of dynein tips the balance in favor of kinesin-2, resulting in movement toward the plus end of the microtubule.

Together, these two studies suggest that a stochastic tug-of-war scenario can explain the behavior of collections of motors. This situation provides a convenient input for higher-level cellular signals that could control the switch. For example, if the frog melanocyte needs to switch its melanosomes from actin to microtubule tracks, it could simply activate a handful of dynein motors (or, conversely, inactivate a handful of myosin V motors). No concerted switch of motor activity is needed, since the rest is handled through the tug-of-war. A particularly attractive feature of the models of Schroeder *et al.* [2] and Hendricks *et al.* [3] is that they highlight the statistical nature of the tug-of-war, where only a handful of

motors are involved. Although cargoes can be propelled by multiple motors, there is no guarantee that all motors are engaged and cooperating at all times. Indeed, recent work by Rogers *et al.* [11] has indicated that multiple kinesin motors rarely cooperate, and instead can often compete with each other.

The fact that the beads in the Schroeder *et al.* [2] study can stall at intersections is particularly revealing behavior. In one sense, it reveals that simple tug-of-war situations can mimic many features of the switching events, and that we do not yet need to invoke more complex models. Mallik and Gross [9] recently argued that pauses in the kinesin- and dynein-driven intraflagellar traffic in *Chlamydomonas* ruled out tug-of-war scenarios, presumably because a tug-of-war should always have a winner that is apparent in an instant. Instead, they favored a concerted switching mechanism. However, Schroeder *et al.* [2] show that stalemates can arise when the forces are carefully balanced, so tug-of-war scenarios may be common when switching tracks or motors within the cell. In a second sense, the pauses in this minimal system could allow for more complex behavior at the filament intersections. For instance, pauses could allow some time for each motor type to 'call in the reserves'. If some myosin V motors have not engaged the track, perhaps due to poor positioning on the bead, they would have additional time to engage and tip the scales in favor of transport along the actin filament. Potentially, within the relatively fluid environment of most vesicle surfaces, every active motor would have an opportunity to engage a track with minimal delay. In these two *in vitro* systems, it may be possible in the future to detect when individual motors engage and detach by detecting small position shifts in the bead or vesicle and thereby determine how such a recruitment process could occur. Clearly, further work is needed to unravel the molecular details as one motor hands off the work to another, but the tug-of-war system outlined by Schroeder *et al.* [2] and Hendricks *et al.* [3] serves as an elegant starting point for these efforts.

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